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DOI:

[10.1016/j.scr.2015.11.007](https://doi.org/10.1016/j.scr.2015.11.007)

*Document Version*

Publisher's PDF, also known as Version of record

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*Citation for published version (APA):*

Miere, C., Wood, V., Kadeva, N., Cornwell, G., Codognotto, S., Stephenson, E., & Ilic, D. (2016). Generation of KCL032 clinical grade human embryonic stem cell line. *Stem Cell Research*, 16(1), 17-19. DOI: 10.1016/j.scr.2015.11.007

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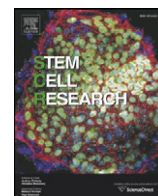
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Lab resource: Stem cell line

# Generation of KCL032 clinical grade human embryonic stem cell line



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## ARTICLE INFO

### Article history:

Received 24 November 2015

Accepted 24 November 2015

Available online 26 November 2015

## ABSTRACT

The KCL032 human embryonic stem cell line was derived from a normal healthy blastocyst donated for research. The ICM was isolated using laser microsurgery and plated on  $\gamma$ -irradiated human foreskin fibroblasts. Both the derivation and cell line propagation were performed in an animal product-free environment and under current Good Manufacturing Practice (cGMP) standards. Pluripotent state and differentiation potential were confirmed by in vitro assays.

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## 1. Resource table

Name of stem cell line	KCL032
Institution	King's College London, London UK
Derivation team	Neli Kadeva, Victoria Wood, Glenda Cornwell, Stefano Codognotto, Emma Stephenson
Contact person and email	Dusko Ilic, email: <a href="mailto:dusko.ilic@kcl.ac.uk">dusko.ilic@kcl.ac.uk</a>
Date archived/stock date	Oct. 10, 2011
Type of resource	Biological reagent: cell line
Sub-type	Human pluripotent stem cell line
Origin	Human embryo
Key marker expression	Pluripotent stem cell markers: NANOG, OCT4, TRA-1-60, TRA-1-81, alkaline phosphatase (AP) activity
Authentication	Identity and purity of line confirmed 1) Jacquet, L., Stephenson, E., Collins, R., Patel, H., Trussler, J., Al-Bedaery, R., Renwick, P., Ogilvie, C., Vaughan, R., Ilic, D., 2013. Strategy for the creation of clinical grade hESC line banks that HLA-match a target population. <i>EMBO Mol. Med.</i> 5 (1), 10–17.
Link to related literature (direct URL links and full references)	doi: 10.1002/emmm.201201973 <a href="http://www.ncbi.nlm.nih.gov/pubmed/23161805">http://www.ncbi.nlm.nih.gov/pubmed/23161805</a> 2) Canham, A., Van Deusen, A., Brison, D.R., De Sousa, P., Downie, J., Devito, L., Hewitt, Z.A., Ilic, D., Kimber, S.J., Moore, H.D., Murray, H., Kunath, T., 2015. The molecular karyotype of 25 clinical-grade human embryonic stem cells lines. <i>Sci. Rep.</i> 5, 17258. doi: 10.1038/srep17258

### Information in public databases

- 3) Ilic, D., Stephenson, E., Wood, V., Jacquet, L., Stevenson, D., Petrova, A., Kadeva, N., Codognotto, S., Patel, H., Semple, M., Cornwell, G., Ogilvie, C., Braude, P., 2012. Derivation and feeder-free propagation of human embryonic stem cells under xeno-free conditions. *Cytotherapy*. 14 (1), 122–128.

doi: 10.3109/14653249.2011.623692

<http://www.ncbi.nlm.nih.gov/pubmed/22029654>

- 4) Stephenson, E., Jacquet, L., Miere, C., Wood, V., Kadeva, N., Cornwell, G., Codognotto, S., Dajani, Y., Braude, P., Ilic, D., 2012. Derivation and propagation of human embryonic stem cell lines from frozen embryos in an animal product-free environment. *Nat. Protoc.* 7 (7), 1366–1381.

doi: 10.1038/nprot.2012.080

<http://www.ncbi.nlm.nih.gov/pubmed/22722371>

KCL032 is a National Institutes of Health (NIH) registered hESC line.

NIH registration number: NIHhESC-14-0266.

[http://grants.nih.gov/stem\\_cells/registry/current.htm?id=652](http://grants.nih.gov/stem_cells/registry/current.htm?id=652)

The hESC line KCL032 is derived under license from the UK Human Fertilisation and Embryology Authority (research license numbers: R0075 and R0133) and also has local ethical approval (UK National Health Service Research Ethics Committee Reference: 06/Q0702/90).

### Ethics

Informed consent was obtained from all subjects and the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the NIH Belmont Report. No financial inducements are offered for donation.

## 2. Resource details

Consent signed	May 26, 2009
Embryo thawed	Jul. 11, 2011
UK stem cell bank deposit	Sep. 13, 2012

\* Corresponding author.

E-mail address: [dusko.ilic@kcl.ac.uk](mailto:dusko.ilic@kcl.ac.uk) (D. Ilic).

(continued on next page)

approval	Reference: SCSC12-37
Sex	Female 46, XX
Grade	Clinical
Disease status	Healthy/unaffected
Karyotype (aCGH)	Gain in region 5p14.3
SNP array	Gain in region 5p14.3 (Canham et al., 2015)
DNA fingerprint	Allele sizes (in bp) of 16 microsatellite markers specific for chromosomes 13, 18 and 21 (Jacquet et al., 2013)
HLA typing	HLA-A2,11; B51,62; Bw4,6; Cw4,9; DR4,13; DR52,53; DQ6,7 (Jacquet et al., 2013)
Viability testing	Pass
Mycoplasma	Negative
Sterility	Pass
Pluripotent markers (immunostaining) (Fig. 1)	NANOG, OCT4, TRA-1-60, TRA-1-81, AP activity
Three germ layers differentiation in vitro (immunostaining) (Fig. 2)	Endoderm: AFP Ectoderm: TUBB3 (tubulin, beta 3 class III) Mesoderm: ACTA2 (actin, alpha 2, smooth muscle)
Sibling lines available	KCL033, KCL034

We generated KCL032 clinical grade hESC line following protocols, established previously (Ilic et al., 2012; Stephenson et al., 2012), and now adapted to cGMP conditions. The expression of the pluripotency markers was tested after freeze/thaw cycle (Fig. 1). Differentiation potential into three germ layers was verified in vitro (Fig. 2).

Molecular karyotyping identified a novel 2.4 Mb gain on chromosome 5p14.3, containing a single gene, *CDH18* (Cadherin-18), that was also present in one of two sibling cell lines, KCL033, but not in KCL034, a third sibling. A duplication of this size has not been reported to date, but its presence in two sibling hESC lines strongly suggests that it was inherited from one of the parents rather than by acquisition during hESC derivation and culture (Canham et al., 2015). The 2498.8 bp gain starts at bp 19,086,546 and ends at bp 21,585,311 as referred to Human Genome Build 38.

We also generated research grade of KCL032 line that is adapted to feeder-free conditions.

### 3. Materials and methods

#### 3.1. Consenting process

We distribute Patient Information Sheet (PIS) and consent form to the in vitro fertilization (IVF) patients if they opted to donate to research

embryos that were stored for 5 or 10 years. They mail signed consent back to us and that might be months after the PIS and consent were mailed to them. For in the meantime new versions of PIS/consent are implemented, we do not send these to the patients or ask them to re-sign; the whole process is done with the version that was given them initially. The PIS/consent documents (FRO-V.6) were created on Dec. 18, 2008. HFEA Code of Practice that was in effect at the time of document creation: Edition 7 – R.4 (<http://www.hfea.gov.uk/2999.html>). The donor couple signed the consent on May 26, 2009. HFEA Code of Practice that was in effect at the time of donor signature: Edition 7 – R.4. HFEA Code of Practice Edition 7 – R.4 was in effect: 02 Oct. 2008–30 Sep. 2009.

#### 3.2. Embryo culture and micromanipulation

Embryo culture and laser-assisted dissection of inner cell mass (ICM) were carried out as previously described in details (Ilic et al., 2012; Stephenson et al., 2012). The cellular area containing the ICM was then washed and transferred to plates containing mitotically inactivated human neonatal foreskin fibroblasts (HFF).

#### 3.3. Cell culture

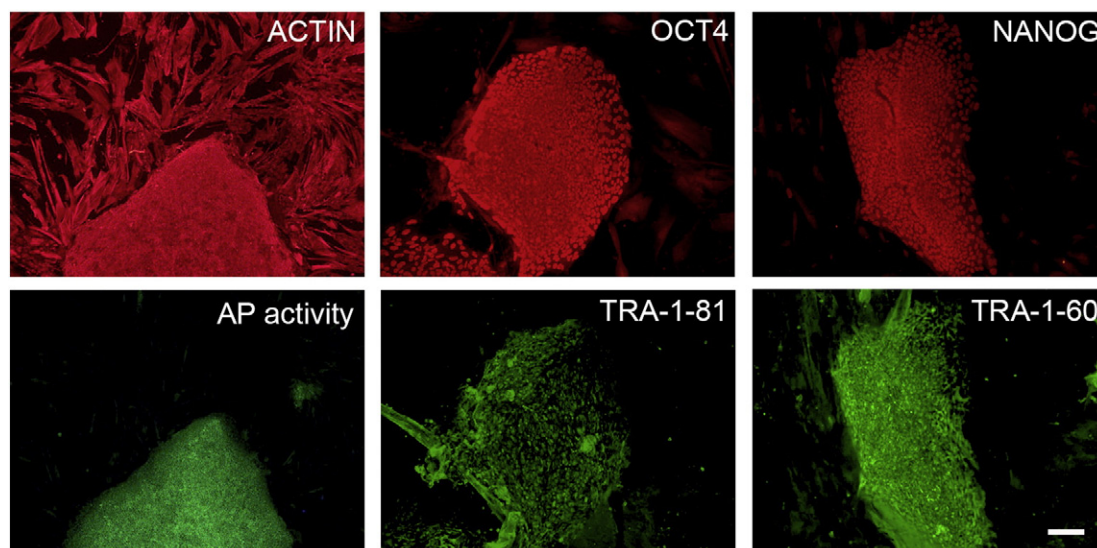
ICM plated on mitotically inactivated HFF were cultured as described (Ilic et al., 2012; Stephenson et al., 2012). TE cells were removed mechanically from outgrowth (Ilic et al., 2007; Ilic et al., 2010). hES colonies were expanded and cryopreserved at the third passage.

#### 3.4. Viability test

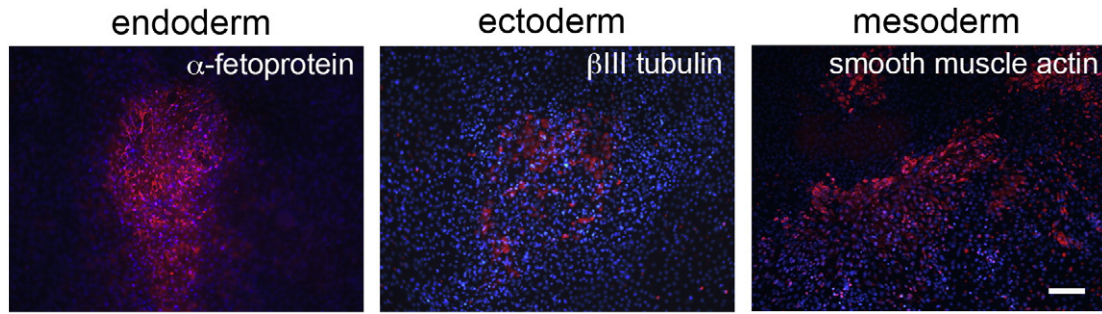
Straws with the earliest frozen passage (p.2–3) are thawed and new colonies are counted three days later. These colonies are then expanded up to passage 8, at which point cells were part frozen and part subjected to standard battery of tests (pluripotency markers, in vitro and in vivo differentiation capability, genetics, sterility, mycoplasma).

#### 3.5. Pluripotency markers

Pluripotency was assessed using two different techniques: enzymatic activity assay [alkaline phosphatase (AP) assay] and immunostaining as described (Ilic et al., 2012; Stephenson et al., 2012).



**Fig. 1.** Expression of pluripotency markers. Pluripotency is confirmed by immunostaining (Oct4, Nanog, TRA-1-60, TRA-1-81) and alkaline phosphatase (AP) activity assay. Actin stress fibers, visualized with rhodamine-phalloidin (red), are present in both feeders and hES cell colonies, whereas AP activity (green) is detected only in hES cells. Scale bar, 50  $\mu$ m.



**Fig. 2.** Differentiation of three germ layers in vitro is confirmed by detection of markers: smooth muscle actin (red) for mesoderm,  $\beta$ -III tubulin (red) for ectoderm and  $\alpha$ -fetoprotein (red) for endoderm. Nuclei are visualized with Hoechst 33342 (blue). Scale bar, 50  $\mu$ m.

### 3.6. Genotyping

DNA was extracted from hES cell cultures using a Chemagen DNA extraction robot according to the manufacturer's instructions. Amplification of polymorphic microsatellite markers was carried out as described (Ilic et al., 2012). Allele sizes were recorded to give a unique fingerprint of each cell line.

### 3.7. Array comparative genomic hybridization (aCGH)

aCGH was performed as described in details (Ilic et al., 2012).

### 3.8. SNP array

SNP array was performed as described in details (Canham et al., 2015).

### 3.9. HLA typing

HLA-A, -B and -DRB1 typing was performed with a PCR sequence-specific oligonucleotide probe (SSOP; Luminex, Austin, TX, USA) hybridization protocol at the certified Clinical Transplantation Laboratory, Guy's and St. Thomas' NHS Foundation Trust and Serco Plc. (GSTS) Pathology (Guy's Hospital, London, UK) as described (Jacquet et al., 2013).

## 4. Author disclosure statement

There are no competing financial interests in this study.

## Acknowledgments

This work was supported by the UK Medical Research Council grants G0701172 and G0801061. We thank Dr. Yacoub Khalaf, Director of the Assisted Conception Unit of Guy's and St. Thomas' NHS Foundation Trust and his staff for supporting the research program. We are especially indebted to Prof. Peter Braude and to the patients who donated embryos.

## References

- Canham, A., Van Deusen, A., Brison, D.R., De Sousa, P., Downie, J., Devito, L., Hewitt, Z.A., Ilic, D., Kimber, S.J., Moore, H.D., Murray, H., Kunath, T., 2015. The molecular karyotype of 25 clinical-grade human embryonic stem cells lines. *Sci. Rep.* 5, 17258.
- Ilic, D., Genbacev, O., Krtolica, A., 2007. Derivation of hESC from intact blastocysts. *Curr. Protoc. Stem Cell Biol.* (Chapter 1: Unit 1A.2).
- Ilic, D., Caceres, E., Lu, S., Julian, P., Foulk, R., Krtolica, A., 2010. Effect of karyotype on successful human embryonic stem cell derivation. *Stem Cells Dev.* 19 (1), 39–46.
- Ilic, D., Stephenson, E., Wood, V., Jacquet, L., Stevenson, D., Petrova, A., Kadeva, N., Codognotto, S., Patel, H., Semple, M., Cornwell, G., Ogilvie, C., Braude, P., 2012. Derivation and feeder-free propagation of human embryonic stem cells under xeno-free conditions. *Cytotherapy* 14 (1), 122–128.
- Jacquet, L., Stephenson, E., Collins, R., Patel, H., Trussler, J., Al-Bedaery, R., Renwick, P., Ogilvie, C., Vaughan, R., Ilic, D., 2013. Strategy for the creation of clinical grade hESC line banks that HLA-match a target population. *EMBO Mol. Med.* 5 (1), 10–17.
- Stephenson, E., Jacquet, L., Miere, C., Wood, V., Kadeva, N., Cornwell, G., Codognotto, S., Dajani, Y., Braude, P., Ilic, D., 2012. Derivation and propagation of human embryonic stem cell lines from frozen embryos in an animal product-free environment. *Nat. Protoc.* 7 (7), 1366–1381.